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Efficient fructose production from plant extracts by immobilized inulinases from *Kluyveromyces marxianus* and *Helianthus tuberosus*

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ABSTRACT

The enzymatic hydrolysis of poly- and oligosaccharides from plants seems like an advantageous approach for sugars production. Two inulinases producing fructose from plant oligosaccharides were isolated from yeast *Kluyveromyces marxianus* and plant *Helianthus tuberosus*. Both enzymes were immobilized on polymeric carriers by using the static adsorption approach. We could save 80.4% of the initial catalytic activity of plant inulinase immobilized on KU-2 cation-exchange resin and 75.5% of yeast enzyme activity adsorbed on AV-17-2P anion-exchange resin. After immobilization, the Km values increased 1.5 and 6 times for enzymes from *K. marxianus* and *H. tuberosus*, respectively. The optimal temperatures for catalysis of both enzymes were increased from 48–50 °C up to 70 °C. The activities of both immobilized enzymes remained unchanged after the 10 cycles of 20-min hydrolysis reaction at 70 °C model batch reactor. Sorbents, native and immobilized enzymes did not exhibit any mutagenic or cytotoxic activity.

1. Introduction

Inulinases (inulase, 2,1-D-fructan-fructohydrolase, E.C. 3.2.1.7) are involved in the carbohydrate metabolism of higher plants and microorganisms. In the food industry, these enzymes are used to obtain sugars with various polymerization degrees like fructose and inulooligosaccharides – the main components of functional nutrition, which reduce the risk of diabetes, dental caries and obesity [1–4]. These sugars have a restorative effect, helping in ethanol removal from the blood, in the iron assimilation by the children, decreasing cholesterol levels in the blood [5–7]. To the date, these enzymes have been thoroughly studied.

For the most inulinases, the influence of the temperature, substrate concentration, pH, the presence of various activators, inhibitors and metal ions in the reaction mixture on catalytic activity of the enzyme have been intensively studied [8–11]. By the contrary, there are only few works with the detailed characteristic of the molecular mechanisms of inulinases stabilization [12,13] or the controlled alteration their structural and functional properties by immobilization on various non-soluble carriers.

Inulinases from *Kluyveromyces marxianus* (63 kDa, radius 6.1 nm) and *Helianthus tuberosus* (65 kDa, radius 5.5 nm) are represented in dimeric form. The association-dissociation process of inulinase and related enzymes plays an important role in the regulation of the metabolism of plants and microorganisms that use inulin, fructooligosaccharides, and fructans as reserve food material [14]. Inulinases and related enzymes are glycoproteins with a glycosylation degree of glycosylation 25–37% [15–17]. The isoelectric points of inulinases are within range of 3.9–4.3 [18].The application of enzymes due to their short life times, unstable structure and extensive separation costs is limited. Improvements in enzyme stability, therefore, can solve these problems and increase their practical applications [19,20]. There have been many approaches to improve the enzyme stability such as immobilization, modification, protein engineering, and medium engineering [21]. Enzyme immobilization represents the attachment or incorporation of enzyme molecules onto or into larges structures, via binding to a carrier, cross-linking and encapsulation [22].

The carrier-bound enzymes are the heterogeneous catalysts and possess a number of significant technological advantages compared to soluble ones. The immobilized enzymes that can be easily separated from the reaction medium, that enables quickly stop the reaction, reuse the catalyst, obtain an enzyme-free product, organize a continuous process flow-type reactors and regulate the speed of the catalysed reaction (or the product yield) by changing the flow rate. As well, due to chemical or physical interaction of the enzyme with a carrier, the key enzyme properties can be altered, for example, optimal pH and temperature [23,24]. In certain cases, immobilization may be associated to enzyme purification and in this way compensate the costs related to the immobilization step [25,26]. Multipoint [27] or multisubunit (in

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